OFFICE OF NAVAL RESEARCH

Contract #N00014-76-C-0146

Task No. NR 204005

Annual Report No. 2

30 September 1977

Title: Schistosome Materials for Vaccine Development

BACKGROUND

The immunological, immunochemical and biochemical investigations carried on for several years at the Naval Medical Research Institute have been directed toward development of a vaccine against schistosomiasis. Until recently, there has been little information on which to build. The immunogenicity of the variety of schistosome materials with which the definitive host comes in contact is not known, nor is there much dependable data on the development of protection by hosts against challenge infections.

This means that basic information must be accumulated before a direct attack on the problem of development of a vaccine can be made. Large quantities of schistosome material are required for these basic investigations. These include, but are not limited to, adult schistosomes, eggs, cercariae, schistosomules, cercarial penetration enzymes, and vertebrate and invertebrate host serum and tissues. It has been the function of this contract to supply these materials in large quantity and prepared as needed.

METHODOLOGY

Provision of these materials required the maintenance of <u>Biomphalaria glabrata</u> snails and of Swiss albino mice. Six 20-gallon aquaria supported about 1000 uninfected snails each. From these, 2 to 3 hundred young snails were collected weekly. These were exposed singly to either 1 or 8 to 10 miracidia each,

or otherwise as necessary for different experimental designs. The miracidial source was minced blended livers of mice with infections of 7 to 8 weeks duration. This exposure schedule maintained a constant level of about 1000 infected snails.

More than 1 million cercariae/day were collected from these infected snails 4 days each week and processed as needed or used immediately for experimental work.

Two hundred and fifty to 300 outbred weanling mice were exposed weekly to about 270 cercariae each. These were kept for 7 weeks, sacrificed and perfused for collection of adult worms.

Schistosomules were collected as needed, averaging several hundred per week. This postpenetration stage was recovered from ear skin of mice exposed in vivo to cercariae or after cercarial penetration of dried rat abdominal epidermis.

Fifty to 100 ml of secreted enzyme solution were harvested two to four days each week from cercariae stimulated to secrete in a temperature gradient over skin surface lipid or the active fraction, linolenic acid. Total protein (Lowry method) and enzyme activity against azocoll (dye-coupled collagen) were established spectrophotometrically for each collection.

RESULTS

Schistosome (Schistosoma mansoni) materials have been provided for investigative use as follows: for immunoparasi-

tological investigations at the Naval Medical Research Institute by Drs. Wilton Vannier, Darwin Murrell, Pat Minard, Verne Schinski, David Dean and Richard Jacobson; for EM studies by Drs. Charles Dorsey (NMRI), M. Stirewalt and C. Cousin (BRI); and for enzyme experiments by M. Stirewalt (BRI). These materials included each week: 4 to 6 million cercariae, 20,000 sexed cercariae, 20 to 25 thousand adult worms, about 100 to 300 schistosomules adjusted to demand, about 400 ml of preacetabular gland enzyme secretion, and excretions and secretions (exoantigens) of cercariae as requested.

CURRENT REPORTS AND PUBLICATIONS SUPPORTED IN PART BY

SCHISTOSOME MATERIALS PRODUCED UNDER

N00014-76-C-0146

1. Chen, P. and Dean, D.A. in press. Immune response of guinea pigs to Schistosoma mansoni. II. In vitro blastogenesis and macrophage migration inhibition factor production in response to cercarial, adult worm and egg antigens. American Journal of Tropical Medicine and Hygiene.

2. Dean, D.A. 1977. Role of Schistosoma mansoni eggs in resistance of mice to reinfection. 61st Annual Meeting, Federation of American Societies for Experimental Biology, Chicago, Ill. 3-8 April, 1977. (Abstract).

3. Dean, D.A. in press. Decreased binding of cytotoxic antibody by developing Schistosoma mansoni. Evidence for a surface change independent of host antigen adsorption and membrane turnover. Journal of Parasitology.

4. Dean, D.A., Minard, P., Vannier, W.E. and Murrell, K.D. Submitted. Resistance of mice to secondary infection with Schistosoma mansoni. Evidence for a correlation between egg deposition and worm elimination. American Journal of Tropical Medicine and Hygiene.

5. Dean, D.A., Stirewalt, M.A., Murrell, K.D. and Vannier, W.E. 1977. Mechanisms involved in immune destruction of schistosomes. 52nd Annual Meeting, American Society of Parasitologists, Las Vegas, Nevada, 14-19 August, 1977. (Abstract).

6. Dean, D.A. and Strong, D.M. 1977. Improved assay for monocyte chemotaxis using frozen stored responder cells. Journal of Immunological Methods 14: 65-72.

7. Dorsey, C.H. and Stirewalt, M.A. in press. Schistosoma mansoni: localization of calcium-detecting reagents in electron-lucid areas of specific preacetabular gland granules. Zeitschrift fur Parasitenkunde.

8. Hussain, R., Vannier, W.E. and Murrell, K.D. Submitted. Schistosoma mansoni: hypersensitivity to antigens. II. Gel filtration studies of allergens from adult worms. Experimental Parasitology.

9. Minard, P., Dean, D.A., Vannier, W.E. and Murrell, K.D. Submitted. Effect of immunization on migration of Schistosoma mansoni through lungs. American Journal of Tropical Medicine and Hygiene.

10. Minard, P., Dean, D.A., Jacobson, R.H., Vannier, W.E. and Murrell, K.D. Submitted. Immunization of mice with cobalt-60 irradiated Schistosoma mansoni cercariae. American Journal of Tropical Medicine and Hygiene.

11. Minard P., Murrell, K.D. and Stirewalt, M.A. 1977. Proteolytic, antigenic and immunogenic properties of Schistosoma mansoni cercarial secretion material. American Journal of Tropical Medicine and Hygiene, 26, 491-499.

12. Murrell, K.D., Vannier, W.E. and Minard, P. 1977. Extraction and partial purification of surface-associated antigen of Schistosoma mansoni. Federation Proceedings. 36(3): 1058 (Abstract).

13. Murrell, K.D., Vannier, W.E., Minard P. and Schinski, V.D. 1977. Schistosoma mansoni: extraction and partial characterization of membrane antigens using an assay based on competitive inhibition of human antibodies binding to schistosomules. Experimental Parasitology 41: 446-463.

14. Schinski, V.D., Clutter, W.C. and Murrell, K.D. 1976. Enzyme and ^{125}I- labelled anti-immunoglobulin assays for the study of schistosome antigens and human immunological responses. American Journal of Tropical Medicine and Hygiene, 25: 824-831.

15. Stirewalt, M.A. 1976. Activity of enzymes secreted by cercariae of Schistosoma mansoni. Program and Abstracts of the 51st Meeting of the American Society of Parasitologists (Abstract).

16. Stirewalt, M.A. in press. Quantitative collection and proteolytic activity of preacetabular gland enzyme(s) of cercariae of Schistosoma mansoni. american Journal of Tropical Medicine and Hygiene.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Required numbers of eggs, miracidia, cercariae, schistosomules, worms, infected mouse serum and penetration enzyme were produced and supplied in support of the studies of 9 investigators.

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